

# Epidermal Langerhans Cells from Normal Human Skin Bind Monomeric IgE via FcεRI

By Binghe Wang,\* Armin Rieger,\* Oliver Kilgus,\* Kenichi Ochiai,† Dieter Maurer,\* Dagmar Födinger,\* Jean-Pierre Kinet,† and Georg Stingl\*

From the \*Department of Dermatology I, Division of Cutaneous Immunobiology, University of Vienna Medical School, A-1090 Vienna, Austria; and the †Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20892

## Summary

Human epidermal Langerhans cells (LC) bearing IgE are found in disease states associated with hyperimmunoglobulinemia E. When studying the mechanism(s) underlying this phenomenon, immunohistology revealed that a majority of epidermal LC from normal skin of healthy individuals can specifically bind monomeric IgE. IgE binding to LC could neither be prevented by preincubation of the tissue with monoclonal antibodies (mAb) against either FcεRII/CD23 or FcγRII/CD32, nor by the addition of lactose. However, binding could be entirely abrogated by preincubation with the anti-FcεRIα mAb 15-1, which interferes with IgE binding to FcεRIαγ transfectants. These observations indicated that IgE binding to epidermal LC is mediated by FcεRI rather than by CD23, CD32, or the D-galactose-specific IgE-binding protein. This assumption gained support from our additional findings that: (a) the majority of LC exhibited distinct surface immunolabeling with the anti-FcεRIα mAbs 15-1 and 19-1, but not with any of eight different anti-FcεRII/CD23 mAbs; and (b) transcripts for the α, β, and γ chains of FcεRI could be amplified by polymerase chain reaction from RNA preparations of LC-enriched, but not of LC-depleted, epidermal cell suspensions. In view of the preeminent role of FcεRI crosslinking on mast cells and basophils in triggering the synthesis and release of mediators of allergic reactions, the demonstration of this receptor on epidermal LC may have important implications for our understanding of allergic reactions after epicutaneous contact with allergens.

Several years ago, Bruynzeel-Koomen et al. (1) detected IgE-bearing Langerhans cells (LC)<sup>1</sup> in the skin of atopic dermatitis (AD) patients. This finding was confirmed by other investigators (2, 3) and was considered to be specific for AD. In 1988, Bruynzeel-Koomen et al. (4) showed that LC-enriched epidermal cells from AD patients can form rosettes with IgE-coated SRBC and that this phenomenon could be prevented by preincubation of the epidermal cells with either myeloma IgE or the mAb BB10 directed against the Fc-IgE receptor of eosinophils, platelets, and macrophages. Together with the observation that stimulation of LC-enriched epidermal cells from nonatopics with IL-4 and/or IFN-γ results in anti-FcεRII/CD23 reactivity of LC (5), this finding led to the idea that LC from AD patients bind IgE via FcεRII/CD23

induced by cytokines uniquely released in the AD skin microenvironment. Evidence contradicting this concept came from the recent finding that IgE<sup>+</sup> LC are also found in other disease states associated with hyperimmunoglobulinemia E (6). Based on this observation, we reasoned that the IgE-binding capacity of LC may not be limited to those of diseased skin, but rather be an intrinsic property of these cells. Thus, this study was undertaken to test whether epidermal LC from normal skin of nonatopic persons could bind IgE and, if so, to unravel the nature of the IgE-binding structure.

## Materials and Methods

### Specimen Collection

Full- or split-thickness skin biopsies from clinically normal-appearing areas of 12 nonatopics were obtained during elective surgery and from autopsy specimens using a dermatome (Davol/Simon, Cranston, RI).

<sup>1</sup> Abbreviations used in this paper: AD, atopic dermatitis; EA, egg albumin; eBP, IgE-binding protein; LC, Langerhans cell; PLP, paraformaldehyde-lysine-periodate; WM, washing medium.

## Preparation of Tissue and Cells

**Frozen Sections.** For light microscopic immunolabeling studies, 5- $\mu$ m cryostat sections of snap-frozen (liquid nitrogen) full-thickness skin biopsies were prepared and acetone fixed for 10 min at 4°C.

For ultrastructural investigations, skin biopsy specimens were cut into small cubes and immediately fixed in paraformaldehyde-lysine-periodate (PLP) for 5 h at room temperature (7). The tissue was then transferred to PBS/10% DMSO for 1 h at 20°C, snap frozen, and stored in liquid nitrogen.

**Epidermal Sheets.** Epidermal sheets were obtained from keratomed split-thickness skin by a separation technique using dispase (2.5 U/ml; Boehringer-Mannheim Biochemicals, Mannheim, FRG) (8).

**Epidermal Cell Suspensions.** Keratomed split-thickness skin was placed dermal-side down on a 0.25% trypsin (Difco Laboratories, Detroit, MI)/PBS solution for 60 min at 37°C. Epidermal sheets were peeled from the underlying dermis and transferred to washing medium (WM: RPMI 1640 [Gibco Ltd., Paisley, Scotland] supplemented with 10% heat-inactivated FCS [Flow Laboratories, Ltd., Ayrshire, Scotland], 2 mM L-glutamine [Flow Laboratories], and an antibiotic-antimycotic solution [Gibco Ltd.] consisting of 100  $\mu$ g/ml penicillin, 100  $\mu$ g/ml streptomycin, and 0.25  $\mu$ g/ml fungizone) containing 0.025% deoxyribonuclease I (Sigma Chemical Co., St. Louis, MO). After 20–30 min at room temperature, the sheets were vigorously stirred, and the resulting cell suspension (viability, 70–90% by trypan blue exclusion) was passed through gauze to remove large cell clumps.

**LC-enriched Epidermal Cell Suspensions.** Single epidermal cell suspensions were resuspended in DME (Flow Laboratories) and incubated for 45 min at 37°C on collagen (Vitrogen 100; Collagen Corp., Palo Alto, CA) -coated petri dishes resulting in the preferential attachment of basal keratinocytes. Since differentiating keratinocytes are more susceptible to osmotic shock treatment than other epidermal cells, nonadherent cells were agitated for 1 min in a hypotonic PBS/distilled H<sub>2</sub>O (8/2) solution. Dead cells (~80%) were removed by Lymphoprep (Nyegaard, Oslo, Norway) density gradient centrifugation; the interface cells were then collected and resuspended in WM. The viability of this cell suspension was regularly  $\geq$ 90%, and the quality of LC enrichment ranged between 20 and 70% as determined by FITC-OKT6 immunostaining. These cells were either used for immunolabeling or RNA isolation.

**LC-depleted Epidermal Cell Suspensions.**  $1.5\text{--}2 \times 10^7$  unfractionated epidermal cells were resuspended in 5 ml ice-cold WM containing 0.5  $\mu$ g/ml anti-HLA-DR mAb (Becton Dickinson & Co. (BD), Sunnyvale, CA). Cells were incubated for 30 min on ice, washed, and resuspended in 5 ml PBS/1% FCS. This cell suspension was incubated with 50  $\mu$ l goat anti-mouse IgG covalently bound to magnetic polystyrene beads (Dynabeads M-450; Dynal A. S., Oslo, Norway) under constant rotation for 1 h at 4°C. Cells attaching to the beads were pelleted by application of a magnetic field; nonadherent cells (viability, 70–90%;  $\leq$ 0.05% CD1a<sup>+</sup> cells) were collected, washed, and processed for RNA extraction.

**Dermal Cell Suspensions.** Split-thickness skin from which the epidermis had been removed by either dispase or trypsin separation was exposed to 0.5% trypsin/PBS for 10 min at 37°C. Loosened cells were then freed by mechanical agitation, collected, washed, resuspended in WM, and processed for RNA extraction.

**Cell Lines.** Mouse myeloma cells SP 2/0 Ag 14 and Chinese hamster ovary (CHO) cells were obtained from the American Type Culture Collection (Rockville, MD). A T cell hybridoma lacking the  $\zeta$  chain of the TCR (2M2) was a gift of Dr. R. Klausner (National Institutes of Health). The EBV-transformed B cell line RPMI

8866 was provided by Dr. K. Ishizaka (Johns Hopkins University, Baltimore, MD); the KU812 cell line, a leukemic cell line with basophilic characteristics, was a gift from Dr. K. Kishi (Department of Internal Medicine, Niigata University School of Medicine, Niigata, Japan).

## Transfectants

Stable transfectants secreting soluble human  $\alpha$  chain products have been described elsewhere (9). Stable transfectants expressing surface Fc $\epsilon$ RI $\alpha$  $\gamma$  complexes were obtained by cotransfecting CHO cells or 2M2 cells with human Fc $\epsilon$ RI $\alpha$  and  $\gamma$  chain cDNAs using methods similar to those described previously for the transient expression of human Fc $\epsilon$ RI in COS cells (10). Briefly, the cells (10<sup>7</sup>/ml) were electroporated at 260 V, 500  $\mu$ F for introduction of the following DNAs (100  $\mu$ g/ml total): human  $\alpha$  chain cDNA in pCDL-SR $\alpha$ 296 (a gift from K. Arai, DNAX, Palo Alto, CA; 11) and human  $\gamma$  chain cDNA in pBJIneo (a gift from M. Davis, Stanford, CA; 12). The cells were then allowed to recover and placed in Iscove's medium for 2 d before the addition of 500  $\mu$ g/ml neomycin. Resistant cells were selected for expression of Fc $\epsilon$ RI using FITC-IgE and FACS<sup>®</sup> (Becton Dickinson & Co.).

## Antibodies

Key antibody reagents are listed in Table 1. With the exception of the two anti-Fc $\epsilon$ RI $\alpha$  mAbs, all were obtained from commercial sources.

**Production of mAbs against the Human Fc $\epsilon$ RI $\alpha$  Chain.** BALB/c mice were immunized intraperitoneally at 2-wk intervals with  $5 \times 10^6$  transfected CHO cells expressing surface Fc $\epsilon$ RI $\alpha$  $\gamma$  complexes. 3 d after the fourth immunization, a single spleen cell suspension was prepared from the immunized mice and fused to the mouse myeloma cell line SP 2/0 Ag 14 as described (13). Hybridoma supernatants were screened for antibodies binding to  $\alpha$ / $\gamma$ -transfected CHO cells. Hybridomas secreting antibodies binding to untransfected CHO cells were eliminated. Antibody binding to CHO cells was analyzed by FACS<sup>®</sup> after incubation with FITC-conjugated goat anti-mouse IgG. Several reactive clones were obtained and subcloned three times by limiting dilution. Two stable producer clones designated 15-1 (IgG1,k) and 19-1 (IgG2a,k) were further characterized. The two mAbs were purified by using kits (Pierce Chemical Co., Rockford, IL) according to the manufacturer's instructions.

## Single-labeling Immunostaining Procedures

IgE as well as anti-Fc $\epsilon$ R mAb binding to the cellular substrate was visualized with a streptavidin-biotin-peroxidase (ABC) technique as previously described (14).

**IgE-binding Assay.** Cryostat sections were incubated overnight with one of the IgE preparations (Table 1) at 4°C. Sections were then exposed to biotinylated goat anti-human IgE for 1 h at room temperature, washed, and incubated with ABC as indicated by the manufacturer (Dakopatts). Finally, sections were reacted with 3-amino-9-ethylcarbazole (Sigma Chemical Co.) in 0.05 M acetate buffer, pH 5.0, for 5–10 min, washed, and counterstained with hematoxylin.

In other experiments, bound IgE was detected by exposing the sections to one of the mouse anti-human IgE mAbs (Table 1), followed by consecutive incubations with a 1:100 diluted biotinylated sheep F(ab')<sub>2</sub> anti-mouse Ig (Amersham, Buckinghamshire, UK) and ABC. For control purposes, we either omitted the IgE incuba-

**Table 1.** List of Key Antibody Reagents

Reagent type	Reagent name	Species/isotype	Concentration	Working dilution	Source
Ig preparations	IgE (myeloma, semi-purified)	Human	2 mg/ml	1:20,000-200*	Chemicon (Temecula, CA)
	IgE (serum, semi-purified)	Human	2 mg/ml	1:200*	Chemicon
	Chimeric Human IgE (anti-NP) monoclonal purified protein	Mouse/human	1 mg/ml	1:100*	Serotec (Oxford, UK)
	IgG (serum)	Human	13 mg/ml	1:1300*	Chemicon
Anti-human Ig	Anti-human IgE mAb (DAKO-IgE)	Mouse IgG1		1:100*	Dakopatts (Glostrup, Denmark)
	mAb anti-IgE (Dε1)	Mouse IgG2a		1:100*	Immunotech (Marseille, France)
	mAb anti-IgE (Dε2)	Mouse IgG1		1:100*	Immunotech
	Biotinylated anti-human IgE	Goat Ig		1:20*	Vector Laboratories, Inc. (Burlingame, CA)
	Biotinylated anti-human IgG	Goat Ig		1:20	Vector Laboratories, Inc.
Anti-FcεRIα	15-1	Mouse IgG1	280 μg/ml	1:200*, 1:40†	
	19-1	Mouse IgG2a	75 μg/ml	1:60*, 1:10†	
Anti-FcεRII/CD23	BU 38	Mouse IgG1		1:500*	Binding Site Ltd. (Birmingham, UK)
	TÜ 1	Mouse IgG3		1:50*	Cymbus Bioscience Ltd. (Southampton, Hampshire, UK)
	MHM 6	Mouse IgG1		1:50*, 1:50†	Dakopatts
	BB 10	Mouse IgM		1:100*	Pel-Freez Biologicals (Rogers, AR)
	IOB8	Mouse IgG1		1:100*	Immunotech
	HD50	Mouse IgG2b		1:100*	Coulter Clone (Hialeah, FL)
	Leu 20	Mouse IgG1		1:100*	Becton Dickinson & Co.
	BSL/23	Mouse IgG1		1:100*	Serotech
Anti-FcγRII/CD32	IV.3	Mouse IgG2b		1:40†	Medarex (West Lebanon, NH)

\* Staining.

† Blocking.

tion step or replaced it with IgG. In addition, we controlled the purity of the IgE preparations by reacting the IgE-exposed sections with goat anti-human IgG.

For IgE-binding inhibition studies, cryostat sections were preexposed to various anti-FcR mAbs (15-1 [7 μg/ml]; 19-1 [7.5 μg/ml]; MHM6 [8.8 μg/ml]; IV.3 [25 μg/ml]; Table 1) for 4 h at room

temperature before the IgE-binding assay. To determine whether the IgE-binding protein (εBP), a D-galactose-binding lectin (15, 16), is involved in the binding of IgE to skin cells, IgE preparations were premixed with 100 mM α-lactose (Sigma Chemical Co.) before use in the assay.

*Binding Studies with anti-FcεR mAb* Cryostat sections of normal

human skin or epidermal sheets were incubated with 10% normal sheep serum/PBS for 30 min at room temperature, washed, and incubated overnight at 4°C with one of each of the appropriately diluted (in PBS/5% BSA) anti-FcεRI and anti-FcεRII mAbs listed in Table 1. Substrates were then washed and exposed to a 1:100 diluted biotinylated F(ab')<sub>2</sub> sheep anti-mouse Ig (Amersham) for 1 h at room temperature, washed, and subjected to the ABC technique. Controls included the omission of the primary antibody or its replacement with irrelevant, isotype-matched mAb.

#### Double-labeling Immunostaining Procedures

**Characterization of IgE-binding Epidermal Cells.** Cryostat sections were incubated overnight at 4°C with one of each of the IgE preparations (Table 1), washed, and incubated consecutively for 1 h periods at room temperature with: (a) biotinylated goat anti-human IgE (Table 1), (b) Texas red-streptavidin (1:50; Zymed Labs, Inc., San Francisco, CA), and (c) FITC-labeled anti-CD1a (OKT 6; 1:10; Ortho Diagnostic Systems Inc., Raritan, NJ). The sections were mounted and viewed with a fluorescence microscope (Axiophot; Carl Zeiss, Inc., Oberkochen, FRG) using appropriate filter settings.

**Identification of IgE-bearing Dermal Mast Cells.** IgE-exposed cryostat sections were reacted with mouse anti-human IgE/D<sub>e2</sub> mAb (1:20) followed by a 1:20 diluted rhodamine-conjugated goat F(ab')<sub>2</sub> anti-mouse IgG (Immunotech) and counterstained with FITC-avidin (1:50; Zymed Laboratories) (17).

**Characterization of Anti-FcεRI-reactive Epidermal Cells.** Cryostat sections were labeled with anti-FcεRIα mAb (Table 1) using the ABC technique. After PBS washes, the substrates were incubated at room temperature with 10% normal mouse serum in PBS for 30 min and counterstained with FITC-OKT6 (1:10) for 1 h.

Additionally, we searched for surface expression of FcεRIα on LC by two-color flow cytometry. LC-enriched epidermal cell suspensions were preincubated with human gamma globulin (5 mg/ml; Beriglobin S; Behring, Marburg, FRG), and were then incubated with either mAb 15-1, OKT6, or with an irrelevant control mAb (all purified IgG1; 10 μg/ml). Thereafter, cells were reacted with affinity-purified, biotinylated goat anti-mouse IgG1 (5 μg/ml; gift of Dr. W. Knapp, Institute of Immunology, University of Vienna Medical School, Austria) followed by streptavidin-PE (BD). All samples were then exposed to FITC-anti-HLA-DR (2.5 μg/ml; BD). Flow cytometric analysis was performed on a FACScan® (BD). List mode data of 10<sup>4</sup> epidermal cells gated for HLA-DR positivity were recorded and individual histograms displayed as cell number versus PE fluorescence intensity.

**Identification of FcεRI-bearing Dermal Mast Cells.** After an overnight incubation with the anti-FcεRIα mAb, sections were reacted with rhodamine-conjugated goat F(ab')<sub>2</sub> anti-mouse IgG (1:20, Immunotech) and counterstained with FITC-avidin (1:50).

#### Immunoelectronmicroscopic Studies

10–15-μm cryostat sections of PLP-fixed tissue were subjected to an immunogold staining procedure using the diffusion (“preembedding”) method (18). Briefly, sections were quenched in PBS/1% egg albumin (EA; Sigma Chemical Co.)/0.005% saponin (Sigma Chemical Co.; 19) and then incubated with mAb 15-1 (1:20 in PBS/1% EA/0.005% saponin) for 12 h at 4°C. After washing, the sections were reacted with a 1:5 diluted goat anti-mouse IgG-gold conjugate (5 nm; Amersham), washed, and fixed with 1.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. After incubation in veronal acetate-buffered osmium and 0.5% aqueous uranylacetate, the sections were dehydrated and embedded in Epon 812. In control experiments, the mAb 15-1 was replaced

by an isotype-matched anti-vimentin mAb (1:10; Dakopatts). Ultrathin sections were cut on an “Ultracut” ultramicrotome (Reichert Scientific Instruments, Buffalo, NY) and examined with an electron microscope (1200 XL; Jeol, Tokyo, Japan).

#### RNA Preparation and PCR

Total cellular RNA was extracted from unfractionated, LC-enriched, and LC-depleted epidermal cells, as well as from dermal cells, cell lines, and from a full-thickness skin specimen with guanidinium thiocyanate followed by centrifugation through a CsCl cushion as described (20).

For cDNA synthesis, 2 μg of RNA was denatured at 65°C for 5 min, chilled on ice, and reverse transcribed in a 20-μl reaction volume containing 1× buffer, 10 mM DTT, 300 U Moloney mouse leukemia virus (MMLV) reverse transcriptase (all Gibco-Bethesda Research Laboratories, Gaithersburg, MD), dNTPs (0.5 mM each), 0.5 μl RNase inhibitor, and 0.5 μl dT15 (1 μg/ml) (all Boehringer Mannheim Biochemicals), incubated at 37°C for 90 min, boiled for 5 min, and stored at 4°C.

PCR was carried out using 1 μl cDNA mix in 50-μl reaction volume containing 1× buffer (Clontech, Palo Alto, CA), dNTPs (0.2 mM each; Boehringer Mannheim Biochemicals), 0.4 μM 3' and 5' primers, and 1.5 U Amplitaq DNA Polymerase (Cetus Corp., Norwalk, CT). Samples were overlaid with 50 μl mineral oil (U.S. Biochemical Corp., Cleveland, OH) and cycled in an Intelligent Heating Block (Hybaid, Teddington, UK) 1 min at 94°C, 2 min at 60°C, and 3 min at 72°C for the indicated numbers of cycles. 20 μl of this reaction product was then run on a 1.5% agarose gel and transferred to Nytran N membranes (Schleicher & Schuell, Inc., Dassel, FRG). Blots were prehybridized and hybridized at 68°C as suggested by the supplier. The hybridization probe was a sequence-specific oligonucleotide (2 pM) that had been 3' labeled with terminal transferase (Boehringer Mannheim Biochemicals) and 2.5 μl α[<sup>32</sup>P]dATP (3,000 Ci/mM; Amersham Corp.). After several washes (final stringency, 0.5% SDS/1× SSC, 55°C), the blots were exposed to Kodak XAR5 film in the presence of intensifying screens at -70°C for 12 h.

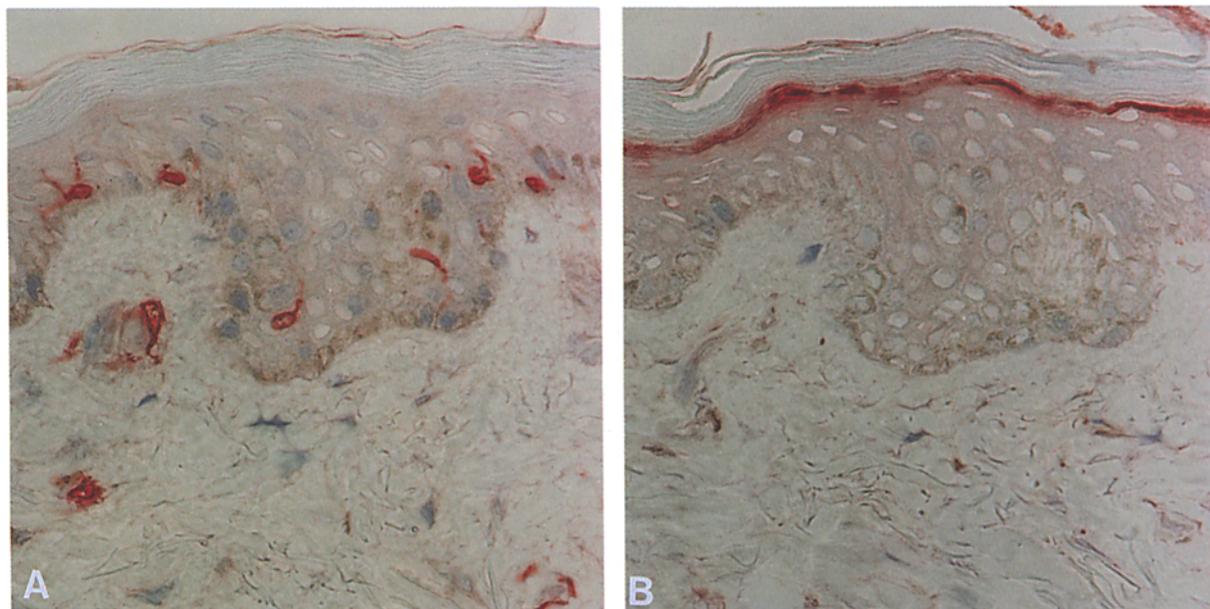
The β-actin primers were purchased from Clontech; other oligonucleotides were synthesized on a Cyclone Plus synthesizer (Milligen, Burlington, MA) following the manufacturer's instruction, and controlled for the absence of degradation by PAGE. Sequences (21–24; J.-P. Kinet, manuscript in preparation) are listed in Table 2.

#### Results

**Demonstration of IgE-binding Cells in Cryostat Sections of Normal Human Skin.** When cryostat sections of normal human skin from 12 nonatopic donors were incubated with various IgE preparations (Table 1) and then reacted with either monoclonal or polyvalent anti-IgE in an indirect immunoperoxidase technique, we regularly observed numerous stained cells in both the epidermis and dermis (Fig. 1 A). The staining intensity was dependent upon the IgE concentration. Optimal results were obtained with 10 or 5 μg/ml, but positive cells were still detectable at 1 μg IgE/ml. Positive cells were not discernible at 0.1 μg IgE/ml. Labeled cells within the epidermis were dendritic in shape and mainly located in a suprabasal position (Fig. 1 A). Stained cells within the dermis were scattered throughout this tissue with a tendency to form clusters around the microvasculature (Fig. 1

**Table 2.** Sequences Used in This Work

Gene	Expected fragment	Sequence	Reference
	<i>bp</i>		
FcεRI, α chain	536		
5' Primer		5'-CTGTTCTTCGCTCCAGATGGCGT-3'	21
3' Primer		5'-TACAGTAATGTTGAGGGGCTCAG-3'	
Internal		5'-CCTTGTACACATCCCAGTTCCTCCAACCAT-3'	
FcεRI, β chain	446		
5' Primer		5'-GGACACAGAAAGTAATAGGAGAG-3'	J.-P. Kinet (manuscript submitted for publication)
3' Primer		5'-GATCAGGATGGTAATTCCCGTT-3'	
FcεRI, γ chain	338		
5' Primer		5'-CCAGCAGTGGTCTTGCTCTTAC-3'	22
3' Primer		5'-GCATGCAGGCATATGTGATGCC-3'	
Tryptase	531		
5' Primer		5'-GGAGCTGGAGGAGCCCGTGA-3'	23
3' Primer		5'-ACCTGGGTAGGAAGCAGTGGTG-3'	
CD23 (FcεRII)	365		
5' Primer		5'-CTGTGGCACTGGGACACCACA-3'	24
3' Primer		5'-TTCAGGGCACGTGTTGCACACA-3'	

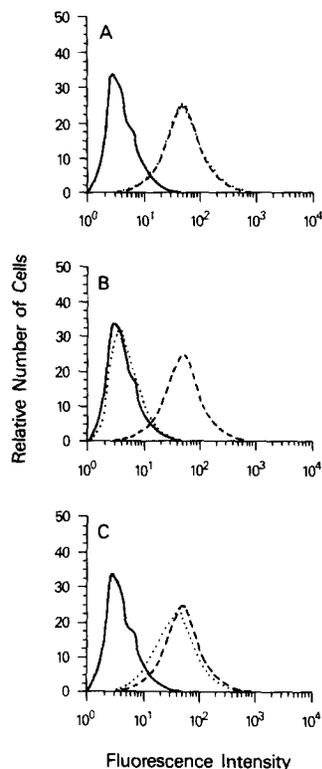


**Figure 1.** Presence of IgE-binding cells within the epidermis and dermis of normal human skin. (A) Detection of stained cells in both the epidermis and dermis after exposure of a cryostat section of normal human skin to monomeric human IgE (5 μg/ml) followed by incubation with goat anti-human IgE in an indirect immunoperoxidase technique. (B) Absence of labeled cells in a cryostat serial section when the anti-IgE reagent was replaced by goat anti-human IgG.

A). No labeled cells were seen when the IgE incubation step was either omitted, substituted for by IgG, or followed by the incubation with the anti-IgG reagent (Fig. 1 B).

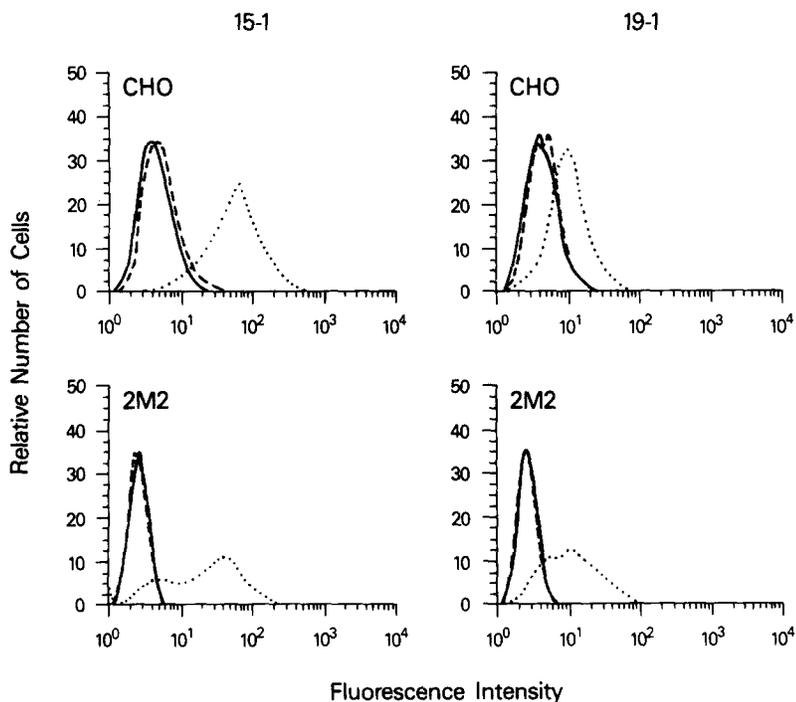
To determine the nature of IgE-binding skin cells, immunofluorescence double-labeling studies were performed on all biopsies. We found that the IgE-binding cells in the epidermis uniformly displayed anti-CD1a reactivity and, thus, represent LC (data not shown). Enumeration of IgE<sup>+</sup> and CD1a<sup>+</sup> cells in 8–10 sequential high-power fields of one section of each biopsy specimen revealed that 45–79% (mean, 64% ± 9% SD) of CD1a-positive cells bound IgE. Within the dermis, some IgE-binding cells, predominantly in the deeper part of this tissue, were FITC-avidin reactive (data not shown) and, thus, represent mast cells (17). IgE-binding cells around the microvessels of the papillary dermis were mostly FITC-avidin negative (data not shown).

**Anti-FcεRIα Chain mAbs.** We have generated and characterized two mAbs, denoted 15-1 and 19-1, against the α chain of FcεRI. These mAbs react with CHO transfectants expressing FcεRIα/γ complexes on their surface but not with nontransfected CHO cells. They also react specifically with mouse T cells (2M2) stably transfected with human FcεRIα and γ chains and express surface α/γ complexes (Fig. 2). Furthermore, the two mAbs reacted strongly and specifically in an ELISA with soluble human α chain purified on an IgE-Sepharose column (data not shown). Taken together, these results indicate that both mAbs react with the α chain of FcεRI. The binding of monomeric IgE to the surface-expressed α chain can be completely inhibited with mAb 15-1 (Fig. 3 B), indicating that the epitope corresponding to 15-1 is close to, or even identical to, the receptor binding site for IgE. By contrast, 19-1 only slightly inhibits the binding of



**Figure 3.** mAb 15-1, but not mAb 19-1, competitively inhibits the binding of mouse IgE to FcεRIα/γ transfectants. Human FcεRIα/γ-transfected CHO cells were preincubated with either 1 μg/ml mouse IgG (A), 1 μg/ml mAb 15-1 (B), or 1 μg/ml mAb 19-1 (C), then reacted with 10 μg/ml FITC-conjugated mouse IgE (.....) and then subjected to FACS<sup>®</sup> analysis. As controls, the results obtained after preincubation with 100 μg/ml of mouse IgE (—) or medium alone (-----) are shown in each panel.

IgE under the same conditions (Fig. 3 C). This may either indicate that the antibody has an affinity too low to compete with IgE, or that it is directed against an epitope unrelated to the receptor binding site. We favor the first explanation



**Figure 2.** mAbs 15-1 and 19-1 react with human FcεRIα/γ transfectants. Human FcεRIα/γ-transfected (.....) or nontransfected (-----) CHO and 2M2 cells were incubated with either mAb 15-1 or mAb 19-1. Cells were then stained with FITC-labeled goat anti-mouse IgG and subjected to FACS<sup>®</sup> analysis. As an additional control, α/γ transfectants were reacted with FITC-conjugated goat anti-mouse IgG only (—).

since a slight inhibition occurs under our experimental conditions (see Fig. 3 C).

**Anti-FcεRIα mAb 15-1 Prevents IgE Binding to Skin Cells.** To identify the nature of the IgE-binding structure on skin cells, we preincubated the sections with reagents known to prevent cellular Ig binding. We found that neither lactose nor the mAbs MHM6 or IV.3 interfere with the observed IgE binding. Thus, it is unlikely that εBP, FcεRII, or FcγRII are responsible for the binding of monomeric IgE to skin cells. In sharp contrast, the anti-FcεRIα mAb 15-1, but not the mAb 19-1, completely inhibited IgE binding to epidermal cells and nearly completely to dermal cells (Fig. 4, A and B).

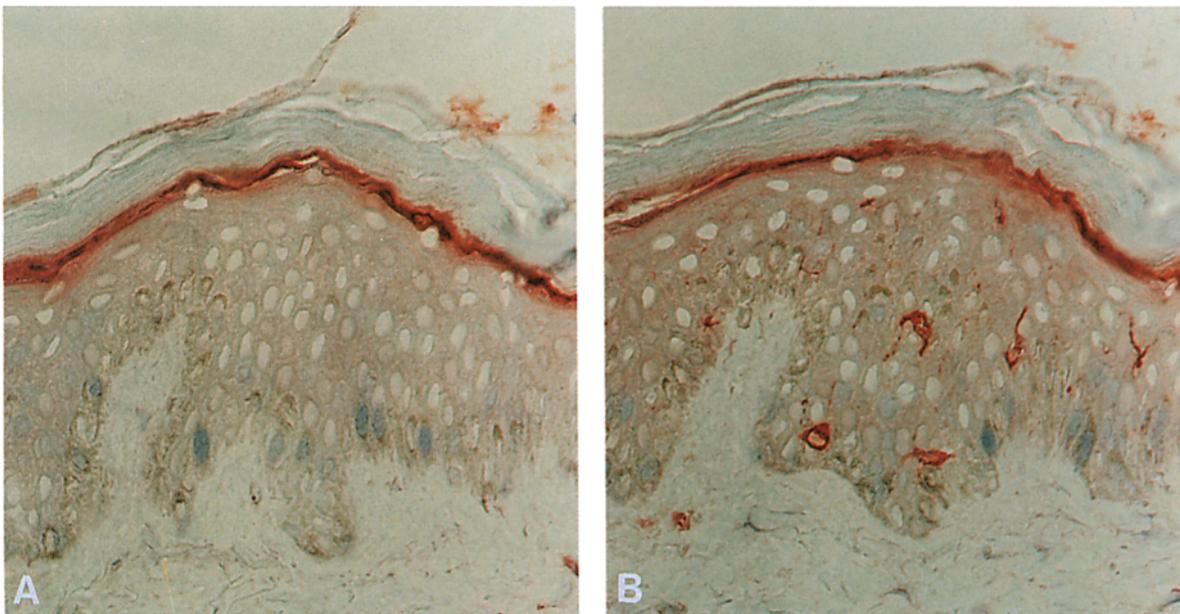
**Anti-FcεRIα mAb, but Not Anti-FcεRII Reagents, Bind to Epidermal Langerhans Cells.** When we screened cryostat skin sections from 12 nonatopic healthy donors for the presence of FcεRII/CD23<sup>+</sup> cells with a panel of eight appropriate reagents (Table 1), both the epidermis and the dermis were consistently devoid of stained cells (data not shown). In contrast, both anti-FcεRIα mAbs regularly gave a staining pattern similar to that seen in our IgE-binding studies. As exemplified by 15-1 immunolabeling on cryostat sections, FcεRIα-positive dermal cells were mostly found around the dermal microvasculature (Fig. 5, A and B) and were occasionally scattered between collagen bundles. Some of these cells were FITC-avidin reactive, thus representing mast cells (data not shown). Within the epidermis, FcεRIα-positive cells exhibited typical LC morphology and topography (Fig. 5, A and B). Their dendritic configuration could be even better appreciated on epidermal sheet preparations (Fig. 5, C and D). Double-

labeling experiments on cryostat sections demonstrated that all FcεRIα-positive epidermal cells are CD1a positive, which confirms their LC nature. Conversely, we found that the vast majority (~80%) of CD1a-bearing epidermal cells displayed anti-FcεRIα reactivity (data not shown).

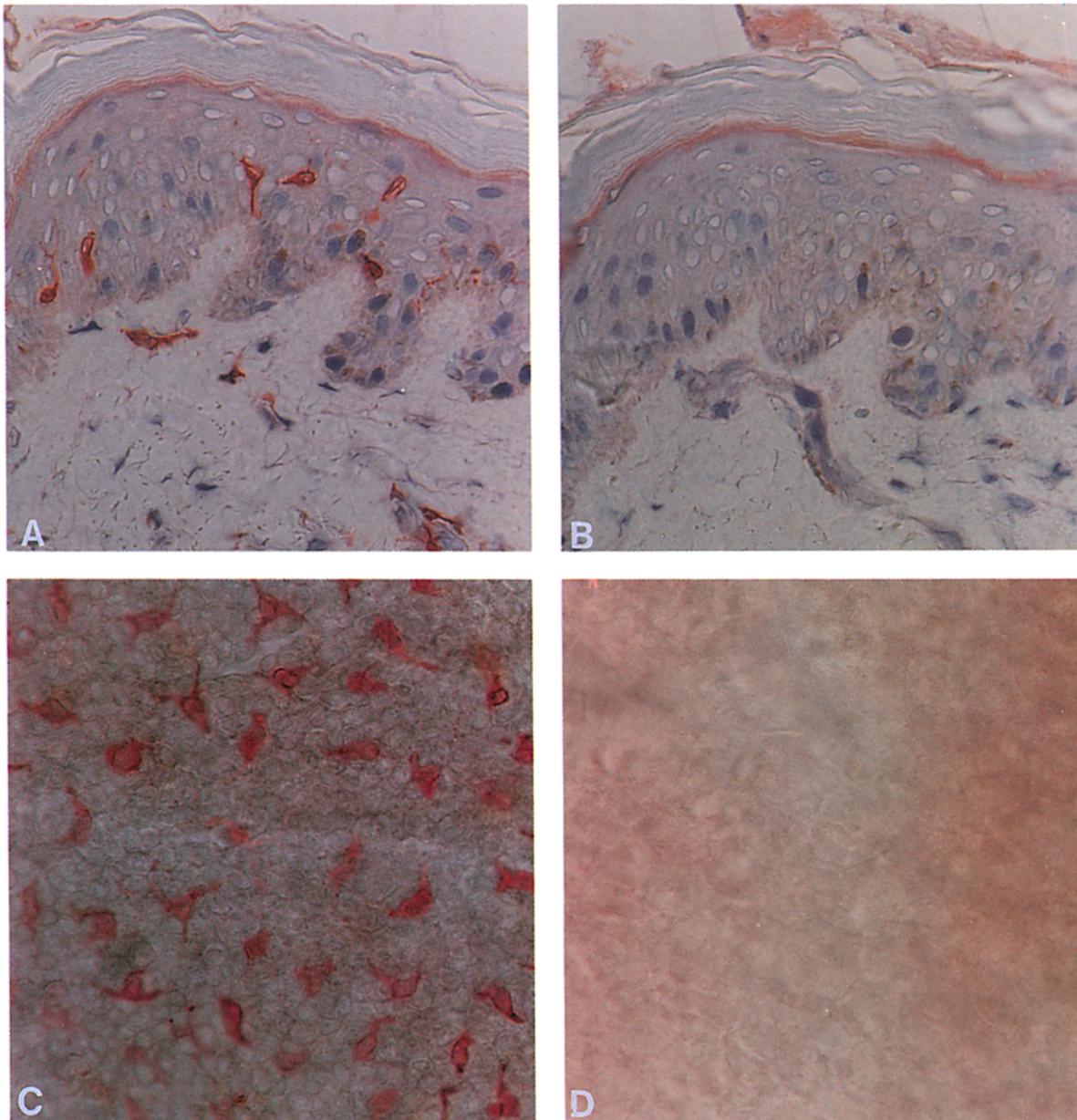
Additionally, flow cytometric analysis of LC-enriched epidermal cell suspensions subjected to a two-color immunolabeling procedure revealed that mAb 15-1 reacts with the surface of HLA-DR/CD1a coexpressing epidermal cells (Fig. 6). In concordance with these data, immunoelectron-microscopic studies showed that LC with their characteristic Birbeck granules are the only anti-FcεRIα-reactive epidermal cells as evidenced by the discontinuous and patchy distribution of 5-nm gold particles along their surfaces (Fig. 7 A). In contrast, the isotype-matched anti-vimentin mAb reacted exclusively with cytoplasmic 10-nm filaments present in LC and melanocytes (Fig. 7 B).

**Presence of FcεRIα, β, and γ Chain Transcripts in LC-enriched, but Not in LC-depleted, Epidermal Cell Suspensions.** Further attempts to identify the IgE-binding structure on LC included the search for FcεR gene expression by skin cells. Total cellular RNA from various cell and tissue preparations was reverse transcribed into cDNA (see Materials and Methods); cDNA equivalent to 100 ng total cellular RNA per sample was used and regularly yielded bands of the expected size when subjected to PCR with β-actin primers (Fig. 8 A). This indicates that each sample contains cDNAs of comparable quality.

Although anti-FcεRII/CD23 mAb immunolabeling studies yielded negative results on LC, FcεRII/CD23 might be present



**Figure 4.** Anti-FcεRIα mAb 15-1 inhibits IgE binding to skin cells. Cryostat serial sections of normal human skin were preincubated with either mAb 15-1 (A) or mAb 19-1 (B), then exposed to monomeric human IgE, and then reacted with biotinylated goat anti-human IgE in an indirect immunoperoxidase technique. Preincubation of sections with mAb 15-1 (A) but not with mAb 19-1 (B) abolishes the IgE-binding capacity of epidermal and dermal cells. ×400.

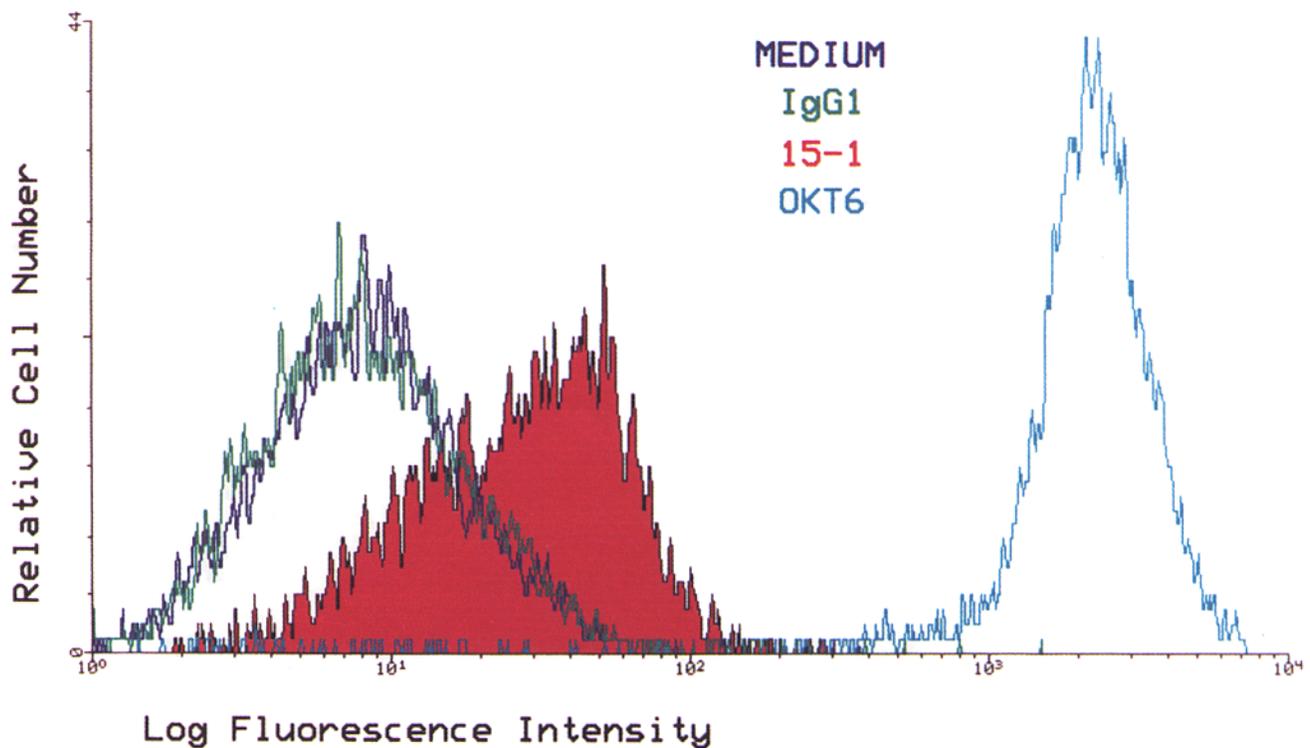


**Figure 5.** Demonstration of FcεRIα-positive cells in cryostat sections and epidermal sheet preparations of normal human skin. Cryostat sections (A and B) or epidermal sheet preparations (C and D) were subjected to either mAb 15-1 (A and C) or to an irrelevant isotype-matched antibody (B and D) in an indirect immunoperoxidase technique. Within the dermis, most 15-1-reactive cells are associated with the microvasculature (A). Within the epidermis, 15-1-positive cells are predominantly located at a suprabasal position (A) and exhibit a pronounced dendritic shape (A and C). × 400.

on LC surfaces in either minute quantities and/or in a configuration inaccessible to these mAbs. Thus, we searched for FcεRII/CD23 mRNA in the various cell preparations using a pair of primers that recognize both alternatively spliced CD23 species (25) and span an intron (26). After 30 PCR cycles, strong bands were generated from RPMI 8866 and slightly weaker bands from KU812 (Fig. 8 B). In contrast, all skin cell samples were negative (Fig. 8 B). 10 additional cycles yielded very faint bands in two of the four LC-enriched epidermal cell preparations, and a more pronounced band was

yielded in the sample of human foreskin (data not shown). Unfractionated and LC-depleted epidermal cell preparations gave negative results (Fig. 8 B). These data make it very unlikely that FcεRII/CD23 is a relevant structure for IgE binding to LC.

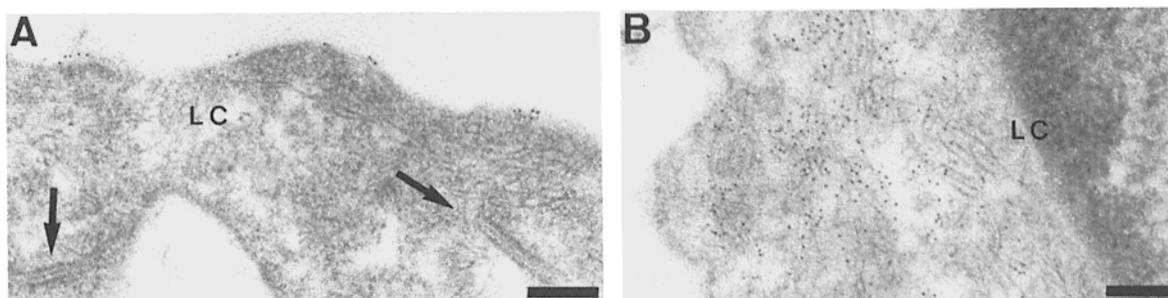
We next searched for the expression of FcεRIα chain mRNA in the various samples. As expected, we found strong signals of the appropriate size in KU812, but not in RPMI 8866, using 25 PCR cycles with FcεRIα chain-specific primers (Fig. 8 C). PCR amplification of cDNA from LC-enriched



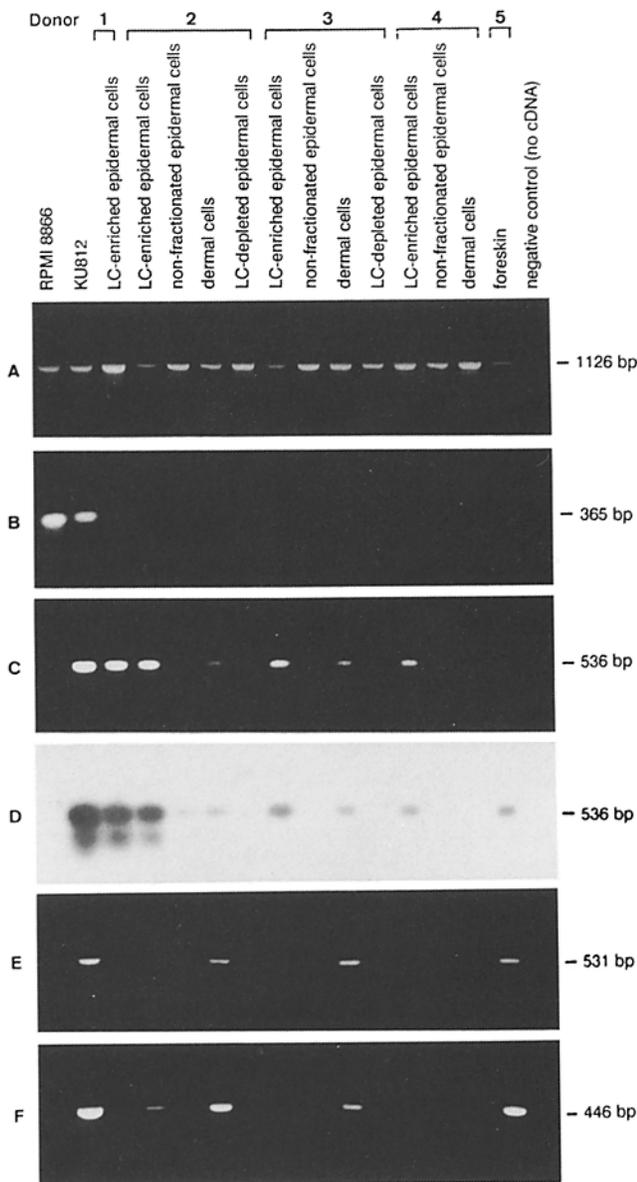
**Figure 6.** Flow cytometric analysis of FcεRIα surface expression on epidermal cells gated for HLA-DR positivity. Overlay histograms represent the reactivity of HLA-DR-positive epidermal cells with either medium alone (*dark blue*), with an irrelevant IgG1 control mAb (*green*), with the anti-FcεRIα mAb 15-1 (*shaded red plot*), or with the mAb OKT6 (*cyan*); mAb binding is visualized by biotinylated goat anti-mouse IgG1 followed by streptavidin-PE, and the logarithmically amplified PE fluorescence intensity is plotted on the abscissa. The homogeneous CD1a expression of the HLA-DR-positive cells confirms their LC nature (*cyan*).

epidermal cell suspensions from four donors yielded bands of equal size and comparable intensity to those seen in the KU812 cell line. Such amplification products were also generated from various dermal and crude epidermal cells, but not from LC-depleted epidermal cells (Fig. 8 C). Note that within a given donor the intensity of signals from LC-enriched epidermal cells was regularly stronger than those from dermal cell samples (containing mast cells) and far exceeded those generated from unfractionated epidermal cells (Fig. 8 C). This pattern of gene expression was entirely reproducible in five

independent experiments. Our contention that the observed bands represent FcεRIα chain-specific mRNA was proven by the findings that: (a) omission of reverse transcriptase yielded no signal (data not shown); (b) the pair of primers spans an intron (J.-P. Kinet, unpublished observation); and (c) the blotted band hybridized with an internal oligonucleotide under stringent conditions (Fig. 8 D). These data strongly suggest that LC, but no other epidermal cells, contain FcεRIα chain transcripts. However, could a few mast cells putatively present in LC-enriched, but not in LC-depleted, epidermal cell sus-



**Figure 7.** Ultrastructural localization of anti-FcεRIα-reactive moieties on the surface of LC. Saponin-treated cryostat sections of PLP-fixed normal human skin were incubated with either mAb 15-1 or an isotype-matched anti-vimentin mAb in an indirect immunogold-labeling technique and then processed for ultrastructural examination. The 15-1 reactivity of LC (identified by its characteristic Birbeck granules) (*arrows*) is evidenced by the patchy and discontinuous distribution of 5-nm gold particles on its surface (*A*). In contrast, anti-vimentin staining results in the selective decoration of LC intermediate-sized filaments with the gold particles (*B*).  $\times 50,000$ ; bars, 0.2  $\mu\text{m}$ .



**Figure 8.** Demonstration of FcεRIα chain mRNA in LC-enriched, but not in LC-depleted, epidermal cells from four different donors. Oligo(dT)-primed cDNA equivalent to 100 ng total cellular RNA was obtained from cell lines RPMI 8866 and KU812, from various skin cell preparations, and from a sample of human foreskin. Subsequent PCR amplification was carried out for the indicated number of cycles using sequence-specific 3' and 5' primers. The reaction product was then run on a 1.5% agarose gel. In the case of the FcεRIα chain, it was blotted onto Nytron N membranes and hybridized with an internal oligonucleotide as described in Materials and Methods. (A) β-actin, 30 cycles; (B) CD23, 30 cycles; (C) FcεRIα chain, 25 cycles; (E) tryptase, 30 cycles; (F) FcεRIβ chain, 35 cycles. The Southern blot of C is shown in D.

pensions account for the observed FcεRIα chain mRNA expression in the LC-containing epidermal cell samples? To test this possibility, PCR was carried out for tryptase, an enzyme that, among skin cells, is considered to be specific for mast cells (27). As shown in Fig. 8 E, tryptase expression is confined to the dermal cell compartment, thus excluding contaminating

mast cells as the source of the observed α chain expression in the LC-enriched samples.

In mast cells, FcεRI is expressed as a heterotetrameric holoreceptor consisting of one α, one β, and two γ chains (28). In transfectants, α and γ chains suffice for surface expression of a high affinity IgE binding site (9). This raises the question whether expression of FcεRIα, β, and γ chain mRNA are linked in cells other than mast cells and basophils. The β chain has recently been sequenced, and its genomic organization was determined (J.-P. Kinet, manuscript in preparation). Using appropriate amplification either from oligo(dt)-primed cDNA or from cDNA generated with a chain-specific 3' primer, we readily detected FcεRIβ chain mRNA in KU812 cells and dermal cells whereas only trace amounts were identified in LC-enriched epidermal cell samples. Unfractionated and LC-depleted epidermal cells were devoid of FcεRIβ chain mRNA (Fig. 8 F). These data indicate that the regulation of the FcεRIβ chain in LC may differ from that in mast cells. In contrast, we found that the γ chain mRNA expression pattern closely follows that of the α chain, suggesting coordinate regulation of expression in both mast cells and LC (data not shown).

## Discussion

This study demonstrates that the majority of resident epidermal LC are capable of binding monomeric IgE. When designing experiments to define the structure responsible for this phenomenon, we considered four candidates: (a) The tetrameric FcεRI, which binds both homologous and heterologous monomeric IgE with high affinity (28); (b) the single-chained FcεRII, which binds IgE with an ~100 times lower affinity than FcεRI (24, 29, 30); (c) the εBP, a β galactoside-specific lectin originally described in a rat basophilic leukemia cell line (15, 16); (d) the single-chained FcγRII/CD32 with its polymorphic cytoplasmic tail, which binds monomeric IgG with very low affinity (28) and which appears to be the only FcγR species expressed by epidermal Langerhans cells (31).

Concerning the last candidate, we were originally concerned that the binding of the various IgE preparations to LC could be due to trace amounts of IgG putatively present in these preparations. However, LC staining was not seen when incubation of the sections with IgE was either substituted for by IgG or followed by incubation with anti-IgG. Additionally, since murine FcγRII are capable of binding IgE (J.-P. Kinet, unpublished results), we preincubated sections with mAb IV.3 directed against the IgG-binding site of CD32 (32). This preincubation step did not prevent IgE binding to LC, a strong argument against the possibility that IgE binds human LC via FcγRII.

Various human myeloma IgE proteins known to be extensively sialylated react with human εBP only when they are pretreated with neuraminidase (16). In the case of polyclonal serum IgE, there is individual variation in the proportion of IgE recognized by εBP depending on the degree of sialylation of different IgE oligosaccharides (33). Our study shows

that epidermal LC bind both myeloma and serum IgE and that the latter binding cannot be competitively blocked by lactose. Thus, we conclude that  $\epsilon$ BP is of little or no relevance for LC IgE binding.

A body of evidence strongly argues against the involvement of Fc $\epsilon$ RII/CD23 in the binding of IgE to resident epidermal LC. First, binding was not blocked with mAb MHM6, which readily inhibits IgE-binding to normal B cells, B cell lines, and promonocytic cell lines (34; A. Rieger, unpublished observations). Second, we failed to detect any epidermal cell immunostaining when cryostat sections of normal human skin were exposed to eight anti-CD23 mAb recognizing different epitopes of this molecule. Although this observation suggests that LC in situ do not bear Fc $\epsilon$ RII/CD23 moieties, our PCR studies, as well as immunoelectron-microscopic studies by Torresani et al. (35), indicate that freshly isolated LC, or at least a subpopulation thereof, express minute quantities of this molecule. Since it is conceivable that the perturbation of the epidermal milieu required for the preparation of single epidermal cell suspensions might trigger events resulting in CD23 gene expression, one cannot safely conclude that the extremely weak CD23 expression by freshly isolated LC is representative of the in situ situation.

Our data are in excellent agreement with the notion that the binding of monomeric IgE to epidermal LC is mediated by the high affinity IgE receptor, Fc $\epsilon$ RI. Strong support for this assumption came from studies with the mAbs 15-1 and 19-1. By FACS<sup>®</sup>, these reagents react specifically with CHO and mouse T cell transfectants expressing Fc $\epsilon$ RI $\alpha\gamma$  complexes on their surfaces, and by ELISA, these mAbs bind strongly and specifically to soluble human Fc $\epsilon$ RI $\alpha$ . Competitive binding studies revealed that mAb 15-1, which interferes with IgE binding to the Fc $\epsilon$ RI $\alpha$  chain expressed on the surface of Fc $\epsilon$ RI $\alpha\gamma$  transfectants, completely inhibits the binding of IgE to LC in cryostat sections of normal human skin. Conversely, mAb 19-1, which only slightly inhibits IgE binding to the transfectants, failed to prevent IgE binding to LC. Finally, light and electron microscopic immunolabeling studies with mAbs 15-1 and 19-1 provided direct evidence for the presence of Fc $\epsilon$ RI $\alpha$  on the surfaces of LC, but no other epidermal cells.

The restricted availability of large specimens of human skin has impeded our efforts to immunochemically characterize the Fc receptor for monomeric IgE on LC. The detection of specific transcripts for the various Fc $\epsilon$ RI chains in RNA

from LC-enriched epidermal cell suspensions indicates that it is a multimeric complex composed of the same polypeptide chains as the Fc $\epsilon$ RI of basophils and mast cells (28). Our additional finding that the relative level of Fc $\epsilon$ RI $\beta$  chain mRNA in LC-enriched epidermal cell suspensions appears to be lower than in dermal cell suspensions may simply be attributable to differences in amplification efficiency. Alternatively, the Fc receptor for monomeric IgE on LC might occur in two configurations: one form identical to that found on mast cells and basophils, and the other form consisting only of  $\alpha$  and  $\gamma$  chains.

This study demonstrates for the first time that LC and certain dermal dendritic cells predominantly located around the dermal microvasculature (36) in healthy human skin can bind monomeric IgE via Fc $\epsilon$ RI $\alpha$ . An important question remains: Does a similar receptor-ligand interaction also occur in pathologic situations in which IgE binds in vivo to LC and other dendritic cells, e.g. AD (1-4)? Recently, we performed IgE-binding studies on acid-treated cryostat sections of lesional skin from one AD patient. This treatment successfully removed in vivo bound IgE. Incubation of these acid-treated sections with monomeric human IgE again led to LC anti-IgE reactivity, which could be blocked by the anti-Fc $\epsilon$ RI $\alpha$  mAb 15-1 (Wang et al., unpublished observations). Should this finding be confirmed with a larger number of AD patients, it would support the concept that Fc $\epsilon$ RI is the critical and biologically relevant IgE-binding molecule on LC of AD patients.

Although signal transduction via the Fc $\epsilon$ RI on LC has yet to be demonstrated, it is attractive to speculate that antigenically or artificially crosslinked IgE on LC may stimulate events in these cells similar to those occurring in mast cells and basophils, e.g., biosynthesis and secretion of arachidonic acid metabolites (37) and cytokines (38, 39). The recent observation by Mudde et al. (40) that IgE-bearing LC are superior to IgE-negative LC in their capacity to present house dust antigens to sensitized T cells, and that this in vitro function correlates with the in vivo presence of a positive delayed patch reaction to house dust antigen, indicates that the Fc $\epsilon$ RI on LC may also play a crucial role in the uptake and processing of allergens by these cells. Should further studies establish or substantiate the role of this receptor in these processes, the Fc $\epsilon$ RI on LC might be an ideal target for topical therapy of allergic skin diseases.

---

We thank Mrs. Regina Holzhauser, Ms. Jutta Maly, and Mr. Heinz Gaudek for excellent technical assistance, and Mrs. Sabine Seizov for carefully typing the manuscript.

This work was supported, in part, by a grant from the Sandoz Research Institute, Vienna, Austria.

Address correspondence to Georg Stingl, Department of Dermatology I, Division of Cutaneous Immunobiology, University of Vienna Medical School, Alser Strasse 4, A-1090 Vienna, Austria.

*Received for publication 30 December 1991 and in revised form 5 February 1992.*

## References

1. Bruynzeel-Koomen, C., D.F. van Wichem, J. Toonstra, L. Berrens, and P.L.B. Bruynzeel. 1986. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch. Dermatol. Res.* 278:199.
2. Barker, J.N.W.N., V.A. Alegre, and D.M. MacDonald. 1988. Surface-bound immunoglobulin E on antigen-presenting cells in cutaneous tissue of atopic dermatitis. *J. Invest. Dermatol.* 90:117.
3. Bieber, T., B. Dannenberg, J.C. Prinz, E.P. Rieber, W. Stolz, O. Braun-Falco, and J. Ring. 1989. Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the IgE serum level. *J. Invest. Dermatol.* 92:215.
4. Bruynzeel-Koomen, C., E.M.M. van der Donk, P.L.B. Bruynzeel, M. Capron, G.C. de Gast, and G.C. Mudde. 1988. Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. *Clin. Exp. Immunol.* 74:137.
5. Bieber, T., A. Rieger, C. Neuchrist, J.C. Prinz, E.P. Rieber, G. Boltz-Nitulescu, O. Scheiner, D. Kraft, J. Ring, and G. Stingl. 1989. Induction of FcεR2/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and γ interferon. *J. Exp. Med.* 170:309.
6. Bieber, T., and O. Braun-Falco. 1991. IgE-bearing Langerhans cells are not specific to atopic eczema but are found in inflammatory skin diseases. *J. Am. Acad. Dermatol.* 24:658.
7. McLean, I.W., and P.K. Nakane. 1974. Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. *J. Histochem. Cytochem.* 22:1077.
8. Kitano, Y., and N. Okada. 1983. Separation of the epidermal sheet by dispase. *Br. J. Dermatol.* 108:555.
9. Blank, U., C. Ra, and J.-P. Kinet. 1991. Characterization of truncated α chain products from human, rat, and mouse high affinity receptor for immunoglobulin E. *J. Biol. Chem.* 266:2639.
10. Miller, L., U. Blank, H. Metzger, and J.-P. Kinet. 1989. Expression of high-affinity binding of human immunoglobulin E by transfected cells. *Science (Wash. DC)*. 244:334.
11. Takebe, Y., M. Seiki, J.-I. Fujisawa, P. Hoy, K. Yokota, K.-I. Arai, M. Yoshida, and N. Arai. 1988. SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type I long terminal repeat. *Mol. Cell. Biol.* 8:466.
12. Lin, A.Y., B. Devaux, A. Green, C. Sagerström, J.F. Elliott, and M.M. Davis. 1990. Expression of T cell antigen receptor heterodimers in a lipid-linked form. *Science (Wash. DC)*. 249:677.
13. Ochiai, K., I. Iwamoto, H. Takahashi, S. Yoshida, N. Nakagawa, H. Tomioka, and S. Yoshida. 1991. Characteristics of an anti-eosinophil monoclonal antibody that recognizes granulocytes from patients with blood eosinophilia but not from subjects without eosinophilia. *Clin. Exp. Immunol.* 84:283.
14. Hsu, S.-M., L. Raine, and H. Fanger. 1981. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* 29:577.
15. Liu, F.-T. 1990. Molecular biology of IgE-binding protein, IgE-binding factors, and IgE receptors. *Crit. Rev. Immunol.* 10:289.
16. Robertson, M.W., K. Albrandt, D. Keller, and F.-T. Liu. 1990. Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. *Biochemistry*. 29:8093.
17. Bergstresser, P.R., R.E. Tigelaar, and M.D. Tharp. 1984. Conjugated avidin identifies cutaneous rodent and human mast cells. *J. Invest. Dermatol.* 83:214.
18. Kerjaschki, D., L. Noronha-Blob, B. Sacktor, and M.G. Farquhar. 1984. Microdomains of distinctive glycoprotein composition in the kidney proximal tubule brush border. *J. Cell Biol.* 98:1505.
19. Ohtsuki, I., R.M. Manzi, G.E. Palade, and J.D. Jamieson. 1978. Entry of macromolecular tracers into cells fixed with low concentrations of aldehydes. *Biol. Cell.* 31:119.
20. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
21. Kochan, J., L.F. Pettine, J. Hakimi, K. Kishi, and J.-P. Kinet. 1988. Isolation of the gene coding for the alpha subunit of the human high affinity IgE receptor. *Nucleic Acids Res.* 16:3584.
22. Küster, H., H. Thompson, and J.-P. Kinet. 1990. Characterization and expression of the gene for the human Fc receptor γ subunit. Definition of a new gene family. *J. Biol. Chem.* 265:6448.
23. Miller, J.S., E.H. Westin, and L.B. Schwartz. 1989. Cloning and characterization of complementary DNA for human trypsinase. *J. Clin. Invest.* 84:1188.
24. Lüdin, C., H. Hofstetter, M. Sarfati, C.A. Levy, U. Suter, D. Alaimo, E. Kilchherr, H. Frost, and G. Delespese. 1987. Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:109.
25. Yokota, A., H. Kikutani, T. Tanaka, R. Sato, E.L. Barsumian, M. Suemura, and T. Kishimoto. 1988. Two species of human Fcε receptor II (FcεRII/CD23): tissue-specific and IL-4-specific regulation of gene expression. *Cell.* 55:611.
26. Suter, U., R. Bastos, and H. Hofstetter. 1987. Molecular structure of the gene and the 5'-flanking region of the human lymphocyte immunoglobulin E receptor. *Nucleic Acids Res.* 15:7295.
27. Irani, A.A., N.M. Schechter, S.S. Craig, G. De Blois, and L.B. Schwartz. 1986. Two types of human mast cells that have distinct neutral protease compositions. *Proc. Natl. Acad. Sci. USA.* 83:4464.
28. Ravetch, J.V., and J.-P. Kinet. 1991. Fc receptors. *Annu. Rev. Immunol.* 9:457.
29. Spiegelberg, H.L. 1984. Structure and function of Fc receptors for IgE on lymphocytes, monocytes, and macrophages. *Adv. Immunol.* 35:61.
30. Kikutani, H., S. Inui, R. Sato, E.L. Barsumian, H. Owaki, K. Yamasaki, T. Kaisho, N. Uchibayashi, R.R. Hardy, T. Hirano, S. Tsunasawa, F. Sakiyama, M. Suemura, and T. Kishimoto. 1986. Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell.* 47:657.
31. Romani, N., A. Lenz, H. Glassel, H. Stössel, U. Stanzl, O. Majdic, P. Fritsch, and G. Schuler. 1989. Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. *J. Invest. Dermatol.* 93:600.
32. Perussia, B., M.A. Cassatella, I. Anegón, and G. Trinchieri. 1989. Antigenic and functional properties of two low-affinity FcγR types: FcγRIII (CD16) on natural killer cells and polymorphonuclear neutrophils and FcεRII (CDw32) on neutrophils and B-cells. In *Leucocyte Typing IV*. W. Knapp, B. Dörken, W.R. Gilks, E.P. Rieber, R.E. Schmidt, H. Stein, and A.E.G. Kr. von dem Borne, editors. Oxford University Press, New York. 590-595.
33. Robertson, M.W., and Liu, F.-T. 1991. Heterogeneous IgE glycoforms characterized by differential recognition of an en-

- dogenuous lectin (IgE-binding protein). *J. Immunol.* 147:3024.
34. Yukawa, K., H. Kikutani, H. Owaki, K. Yamasaki, A. Yokota, H. Nakamura, E.L. Barsumian, R.R. Hardy, M. Suemura, and T. Kishimoto. 1987. A B cell-specific differentiation antigen, CD23, is a receptor for IgE (FcεR) on lymphocytes. *J. Immunol.* 138:2576.
  35. Torresani, C., G.C. Manara, C. Ferrari, and G. de Panfilis. 1991. Immunoelectron microscopic characterization of a subpopulation of freshly isolated epidermal Langerhans cells that reacts with anti-CD23 monoclonal antibody. *Br. J. Dermatol.* 124:533.
  36. Headington, J.T., and R. Cerio. 1990. Dendritic cells and the dermis. *Am. J. Dermatopathol.* 12:217.
  37. Parker, C.W. 1987. Lipid mediators produced through the lipoxigenase pathway. *Annu. Rev. Immunol.* 5:65.
  38. Plaut, M., J.H. Pierce, C.J. Watson, J. Hanley-Hyde, R.P. Nordan, and W.E. Paul. 1989. Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. *Nature (Lond.)* 339:64.
  39. Wodnar-Filipowicz, A., C.H. Heusser, and C. Moroni. 1989. Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature (Lond.)* 339:150.
  40. Mudde, G.C., F.C. van Reijssen, G.J. Boland, G.C. de Gast, P.L.B. Bruijnzeel, and C.A.F.M. Bruijnzeel-Koomen. 1990. Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 69:335.